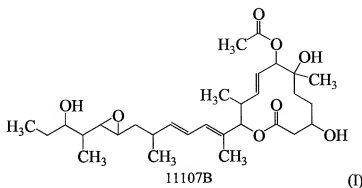
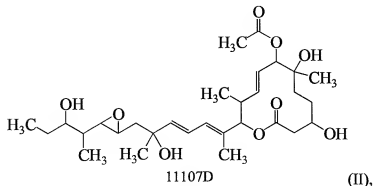


AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A DNA participating in biological transformation of a macrolide compound (hereinafter referred to as a macrolide compound 11107B) represented by the formula (I):



into a 16-position hydroxy macrolide compound represented by the formula (II):



the DNA being an isolated and pure DNA comprising a DNA encoding a protein having 16-position hydroxylating enzymatic activity ~~or ferredoxin, partly or entirely or its variant~~ which is characterized by the following (a), (b), or (c):

(a) a DNA encoding a protein having the enzymatic activity to hydroxylate the 16-position of the macrolide compound 11107B, wherein the DNA is selected from the group consisting of (1) a continuous nucleotide sequence from base 1322 to base 2548 of SEQ ID NO: 1; (2) a continuous nucleotide sequence from base 420 to base 1604 of SEQ ID NO: 2; and a continuous nucleotide sequence from base 172 to base 1383 of SEQ ID NO: 3;

(b) a DNA which has a nucleotide sequence having 90% or more identity with the DNA described in (a);

(c) a DNA encoding a protein having the same amino acid sequence as the protein encoded by the DNA described in (a) or (b) though it does not have 90% or more identity with the DNA described in (a) because of the degeneracy of a gene codon.

2. (Canceled)

3. (Withdrawn, Currently Amended) A protein encoded by the DNA as claimed in Claim [[2]] 1.

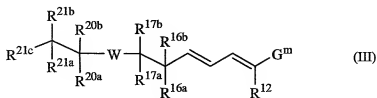
4. (Currently Amended) A self-replicative or integrating replicative recombinant plasmid carrying the DNA as claimed in Claim 1 [[2]].

5. (Original) A transformant into which the recombinant plasmid as claimed in Claim 4 transforms.

6. (Withdrawn, Currently Amended) A method of isolating a DNA encoding a protein having enzymatic activity in hydroxylating the 16-position of the macrolide compound 11107B, the method characterized by using the DNA as claimed in Claim [[2]] 1 or a DNA constituted of a part of the DNA as a probe or a primer.

7-11. (Canceled)

12. (Withdrawn, Currently Amended) A method of producing a 16-position hydroxy macrolide compound, the method comprises the steps of culturing the transformant as claimed in Claim 5 or 10 in a medium; bringing the proliferated transformant into contact with a macrolide compound represented by the formula (III):



(wherein W represents R¹⁸ .or. ;

R¹², R^{16b}, R^{17a}, R^{17b}, R¹⁸, R^{20a}, R^{20b}, R^{21a} and R^{21b}, which may be the same as or different from, respectively represent:

- (1) hydrogen atom;
- (2) a C₁₋₂₂ alkyl group which may have a substituent;
- (3) -OR (wherein R represents:
 - 1) hydrogen atom; or
 - 2) a C₁₋₂₂ alkyl group;
 - 3) a C₇₋₂₂ aralkyl group;
 - 4) a 5-membered to 14-membered heteroaryloxyalkyl group;
 - 5) a C₂₋₂₂ alkanoyl group;
 - 6) a C₇₋₁₅ aroyl group;
 - 7) a C₃₋₂₃ unsaturated alkanoyl group;

8) -COR^{co} (wherein R^{co} represents:

8-1) a 5-membered to 14-membered heteroaryloxyaryl group;

8-2) a C₁₋₂₂ alkoxy group;

8-3) an unsaturated C₂₋₂₂ alkoxy group;

8-4) a C₆₋₁₄ aryloxy group;

8-5) a 5-membered to 14-membered heteroaryloxy group; or

8-6) a 3-membered to 14-membered nitrogen-containing non-aromatic heterocyclic group, each of which may have a substituent);

9) a C₁₋₂₂ alkylsulfonyl group;

10) a C₆₋₁₄ arylsulfonyl group; or

11) -SiR^{s1}R^{s2}R^{s3}, (wherein R^{s1}, R^{s2} and R^{s3}, which may be the same as or different from, respectively represent a C₁₋₆ alkyl group or a C₆₋₁₄ aryl group), each of which may have a substituent);

(4) a halogen atom; or

(5) -R^M-NR^{N1}R^{N2}, {wherein R^M represents a single bond or -O-CO-; and R^{N1} and R^{N2}

1) may be the same as or different from, respectively represent:

1-1) hydrogen atom; or

1-2)

(i) a C₁₋₂₂ alkyl group;

(ii) an unsaturated C₂₋₂₂ alkyl group;

(iii) a C₂₋₂₂ alkanoyl group;

(iv) a C₇₋₁₅ aroyl group;

- (v) an unsaturated C₃₋₂₃ alkanoyl group;
- (vi) a C₆₋₁₄ aryl group;
- (vii) a 5-membered to 14-membered heteroaryl group;
- (viii) a C₇₋₂₂ aralkyl group;
- (ix) a C₁₋₂₂ alkylsulfonyl group; or
- (x) a C₆₋₁₄ arylsulfonyl group, each of which may have a substituent, or

2) and R^{N1} and R^{N2} may be combined with the nitrogen atom to which they bound, to form a 3-membered to 14-membered nitrogen-containing non-aromatic heterocyclic group}, provided that

R^{21a} and R^{21b} may be combined with each other to form (i) a ketone structure (=O) or (ii) an oxime structure {=NOR^{ox} (wherein R^{ox} represents a C₁₋₂₂ alkyl group, an unsaturated C₂₋₂₂ alkyl group, a C₆₋₁₄ aryl group, a 5-membered to 14-membered heteroaryl group or a C₇₋₂₂ aralkyl group, each of which may have a substituent))};

R^{16a} represents hydrogen atom;

R^{21c} represents:

- (1) hydrogen atom; or
- (2)



(wherein R^{22a}, R^{22b} and R^{22c}, which may be the same as or different from, respectively represent:

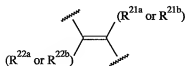
- 1) hydrogen atom;
- 2) a C₁₋₆ alkyl group;

3) -OR (wherein R has the same meaning as the above);

4) $-R^M-NR^{N1}R^{N2}$ (wherein R^M , R^{N1} and R^{N2} have the same meanings as the above); or

5) a halogen atom, or

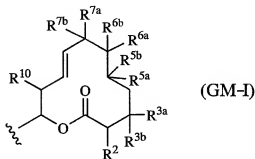
any one of R^{21a} and R^{21b} may be combined with any one of R^{22a} and R^{22b} to form the partial structure;



); and

G^m represents:

(1) a group represented by the formula (GM-I):



(GM-I)

{wherein

R^2 and R^{10} , which may be the same as or different from , respectively represent hydrogen atom or a C_{1-22} alkyl group;

R^{3a} , R^{3b} , R^{5a} , R^{5b} , R^{6a} and R^{6b} , which may be the same as or different from, respectively represent:

1) hydrogen atom;

2) hydroxyl group;

3)

3-1) a C₁₋₂₂ alkyl group;

3-2) a C₁₋₂₂ alkoxy group;

3-3) a C₆₋₁₄ aryloxy group;

3-4) a 5-membered to 14-membered heteroaryloxy group;

3-5) a C₂₋₂₂ alkanoyloxy group;

3-6) a C₇₋₁₅ aroyloxy group;

3-7) a C₃₋₂₃ unsaturated alkanoyloxy group;

3-8) -OCOR⁶⁰ (wherein R⁶⁰ has the same meaning as the above);

3-9) a C₁₋₂₂ alkylsulfonyloxy group;

3-10) a C₆₋₁₄ arylsulfonyloxy group; or

3-11) -OSiR^{s1}R^{s2}R^{s3} (wherein R^{s1}, R^{s2} and R^{s3} have the same meanings as the above),

each of which may have a substituent;

4) a halogen atom; or

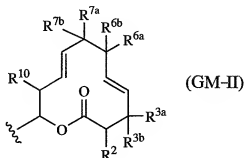
5) -R^M-NR^{N1}R^{N2} (wherein R^M, R^{N1} and R^{N2} have the same meanings as the above); or

R^{5a} and R^{5b} may be combined with each other to form a ketone structure (=O); or

R^{6a} and R^{6b} may be combined with each other to form a spirooxysilanyl group or an exomethylene group; or

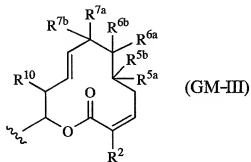
R^{7a} and R^{7b}, which may be the same as or different from, respectively represent hydrogen atom or -OR^H (wherein R^H represents hydrogen atom, a C₁₋₂₂ alkyl group or a C₂₋₂₂ alkanoyl group));

(2) a group represented by the formula (GM-II):



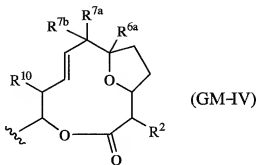
(wherein R^2 , R^{3a} , R^{3b} , R^{6a} , R^{6b} , R^{7a} , R^{7b} and R^{10} have the same meanings as those in the formula (GM-I));

(3) a group represented by the formula (GM-III):



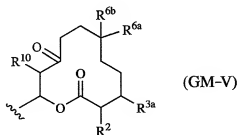
(wherein R^2 , R^{5a} , R^{5b} , R^{6a} , R^{6b} , R^{7a} , R^{7b} and R^{10} have the same meanings as those in the formula (GM-I));

(4) a group represented by the formula (GM-IV):



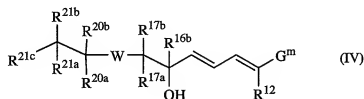
(wherein R^2 , R^{6a} , R^{7a} , R^{7b} and R^{10} have the same meanings as those in the formula (GM-I)); or

(5) a group represented by the formula (GM-V):



(wherein R^2 , R^{3a} , R^{6a} , R^{6b} and R^{10} have the same meanings as those in the formula (GM-I))

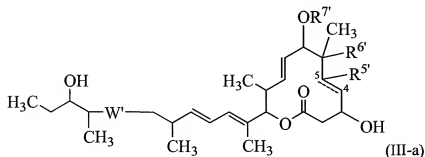
during or after culturing, to convert it into a 16-position hydroxy macrolide compound represented by the formula (IV):



(wherein W, R¹², R^{16b}, R^{17a}, R^{17b}, R^{20a}, R^{20b}, R^{21a}, R^{21b}, R^{21c} and G^m have the same meanings as those in the formula (III)); and then collecting the 16-position hydroxy macrolide compound thus converted.

13. (Canceled)

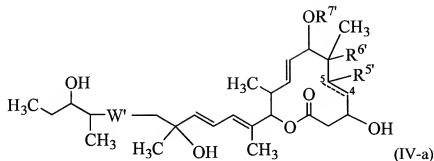
14. (Withdrawn) The production method according to Claim 12, the method comprises the step of converting a compound represented by the formula (III-a):



(wherein $\overset{5}{\text{---}}\overset{4}{\text{---}}$ represents a double bond or a single bond; W' represents a double bond or





; R^{5'} represents hydrogen atom or an acetoxy group; R^{6'} represents hydrogen atom or hydroxyl group; and R^{7'} represents hydrogen atom or acetyl group) into a compound represented by the formula (IV-a):





(wherein $\text{5} \equiv \text{4}$, W' , R^5 , R^6 and R^7 have the same meanings as those in the formula (III-a)).

15. (Withdrawn) The production method according to Claim 14, wherein, in the conversion of the compound of the formula (III-a) into the compound of the formula (IV-a), the compound to be subjected is a compound selected from the group consisting of:

(1) a compound in which $\text{5} \equiv \text{4}$ is a single bond; W' is ; and R^5 , R^6 and R^7 are respectively hydrogen atom;

(2) a compound in which $\text{5} \equiv \text{4}$ is a single bond, W' is ; R^5 and R^6 are respectively hydrogen atom; and R^7 is acetyl group;

(3) a compound in which $\text{5} \equiv \text{4}$ is a single bond, W' is ; R^5 and R^7 are respectively hydrogen atom; and R^6 is hydroxyl group;


(4) a compound in which $5 \equiv 4$ is a single bond, W' is ; R^{5'} is hydrogen atom, R^{6'} is hydroxy group; and R^{7'} is acetyl group;


(5) a compound in which $5 \equiv 4$ is a single bond; W' is a double bond; and R^{5'}, R^{6'} and R^{7'} are respectively hydrogen atom;


(6) a compound in which $5 \equiv 4$ is a single bond; W' is a double bond; R^{5'} and R^{6'} are respectively hydrogen atom; and R^{7'} is acetyl group;


(7) a compound in which $5 \equiv 4$ is a single bond; W' is a double bond; R^{5'} and R^{7'} are respectively hydrogen atom; and R^{6'} is hydroxyl group;

(8) a compound in which $5 \equiv 4$ is a single bond; W' is a double bond; R^{5'} is hydrogen atom; R^{6'} is hydroxy group; and R^{7'} is acetyl group;

(9) a compound in which $5 \equiv 4$ is a double bond; W' is ; R^{5'} and R^{7'} are respectively hydrogen atom; and R^{6'} is hydroxyl group;

(10) a compound in which $5 \equiv 4$ is a double bond; W' is ; R^{5'} is hydrogen atom; R^{6'} is hydroxy group; and R^{7'} is acetyl group;

(11) a compound in which $5 \equiv 4$ is a single bond; W' is ; R^{5'} is acetoxy group; R^{6'} is hydroxyl group; and R^{7'} is hydrogen atom; and

(12) a compound in which $5 \equiv 4$ is a single bond; W' is ; R^{5'} is an acetoxy group; R^{6'} is hydroxyl group; and R^{7'} is acetyl group.

16. (Withdrawn, Currently Amended) Use of the transformant as claimed in Claim 5 or 10 for producing a 16-position hydroxy macrolide compound.

17. (New) The DNA according to claim 1, wherein the DNA comprises bases 1322-2548 of SEQ ID NO: 1.

18. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide comprising amino acids 1-409 of SEQ ID NO: 1.

19. (New) The DNA according to claim 1, wherein the DNA consists of bases 1322-2548 of SEQ ID NO: 1.

20. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide consisting of amino acids 1-409 of SEQ ID NO: 1.

21. (New) The DNA according to claim 1, wherein said identity in (b) and (c) of claim 1 is 95% or more.

22. (New) A DNA comprising

(a) a DNA encoding a protein, wherein the DNA is selected from the group consisting of (1) a continuous nucleotide sequence from base 1322 to base 2548 of SEQ ID NO: 1; (2) a continuous nucleotide sequence from base 420 to base 1604 of SEQ ID NO: 2; and a continuous nucleotide sequence from base 172 to base 1383 of SEQ ID NO: 3;

(b) a DNA which has a nucleotide sequence having 90% or more identity with the DNA described in (a); or

(c) a DNA encoding a protein having the same amino acid sequence as the protein encoded by the DNA described in (a) or (b) though it does not have 90% or more identity with the DNA described in (a) because of the degeneracy of a gene codon.

23. (New) The DNA according to claim 22, wherein said identity in (b) and (c) of claim 22 is 95% or more.

24. (New) The DNA according to claim 1, wherein the DNA comprises bases 420-1604 of SEQ ID NO: 2.

25. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide comprising amino acids 1-395 of SEQ ID NO: 2.

26. (New) The DNA according to claim 1, wherein the DNA consists of bases 420-1604 of SEQ ID NO: 2.

27. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide consisting of amino acids 1-395 of SEQ ID NO: 2.

28. (New) The DNA according to claim 1, wherein the DNA comprises bases 172-1383 of SEQ ID NO: 3.

29. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide comprising amino acids 1-404 of SEQ ID NO: 3.

30. (New) The DNA according to claim 1, wherein the DNA consists of bases 172-1383 of SEQ ID NO: 3.

31. (New) The DNA according to claim 1, wherein the DNA encodes a polypeptide consisting of amino acids 1-404 of SEQ ID NO: 3.